The Lichenologist **51**(1): 7–25 (2019)
doi:10.1017/S0024282918000555

Four new epiphytic species in the *Micarea prasina* group from Europe

Annina LAUNIS, Juha PYKÄLÄ, Pieter VAN DEN BOOM, Emmanuël SÉRUSIAUX and Leena MYLLYS

Abstract: In this study we clarify the phylogeny and reassess the current taxonomy of the *Micarea prasina* group, focusing especially on the *M. byssacea* and *M. micrococca* complexes. The phylogeny was investigated using ITS, mtSSU and *Mcm7* regions from 25 taxa belonging to the *M. prasina* group. A total of 107 new sequences were generated. Data were analyzed using maximum parsimony and maximum likelihood methods. The results reveal five undescribed well-supported lineages. Four of the lineages represent new species described as *Micarea pseudomicrococca* Launis & Myllys sp. nov., *M. czarnotae* Launis, van den Boom, Sérusiaux & Myllys sp. nov., *M. microareolata* Launis, Pykälä & Myllys sp. nov. and *M. laeta* Launis & Myllys sp. nov. In addition, a fifth lineage was revealed that requires further study. *Micarea pseudomicrococca* is characterized by an olive green granular thallus, small cream-white or brownish apothecia lacking the Sedifolia-grey pigment and two types of paraphyses up to 2 µm wide. *Micarea czarnotae* forms a granular, densely granular or continuous olive green thallus, convex to hemispherical apothecia often with the Sedifolia-grey pigment and no crystalline granules in the thallus. *Micarea microareolata* is characterized by a ± pale green areolate thallus (composed of goniocysts), cream-white apothecia lacking the Sedifolia-grey pigment and narrow spores. *Micarea laeta* has a vivid to olive green granular thallus, pale apothecia lacking the Sedifolia-grey pigment and wider spores compared to *M. microareolata*. Descriptions, images and a key are provided for the new species. Crystalline granules are introduced as a novel species-level character for *Micarea*.

Key words: crystalline granules, ITS, lichens, *Mcm7*, mtSSU, taxonomy

Accepted for publication 25 *June* 2018

Introduction

The taxonomy of *Micarea* Fr., a crustose lichen genus in the family *Pilocarpaceae*, is insufficiently known owing to the small number of morphological characters available and difficulties in their interpretation. The genus comprises *c.* 100 species and occurs on all continents (Kirk *et al.* 2008; Coppins 2009). It is best known and most widely collected from Europe where it is widespread and common. However, even after two monographic treatments of the European species of the genus (Coppins 1983; Czarnota 2007), new species and distribution data are frequently published from Europe and Macaronesia (Czarnota & Guzow-Krzemińska 2010; Svensson & Thor 2011; van den Boom & Ertz 2014; Guzow-Krzemińska *et al.* 2016; van den Boom *et al.* 2017) as well as from other lesser known areas (Cáceres *et al.* 2013; Aptroot & Cáceres 2014; Barton & Lendemer 2014; Brand *et al.* 2014; Córdova-Chávez *et al.* 2014; Launis & Myllys 2014; McCarthy & Elix 2016). In many cases, DNA based phylogenies have been necessary for understanding the species diversity.

Recent molecular phylogenies have shown that *Micarea* is paraphyletic (Andersen & Ekman 2005; Sérusiaux *et al.* 2010), even after the introduction of a new genus

A. Launis (corresponding author) and L. Myllys: Botany Unit, Finnish Museum of Natural History, P.O. Box 7, FI-00014 University of Helsinki, Finland. Email: annina.launis@helsinki.fi.
J. Pykälä: Natural Environment Centre, Finnish Environment Institute, P.O. Box 140, FI-00251 Helsinki, Finland.
P. van den Boom: Arafura 16, NL-5691 JA Son, The Netherlands.
E. Sérusiaux: Evolution and Conservation Biology Unit, InBios Research Center, University of Liège, Sart Tilman B22, B-4000 Liège, Belgium.

Brianaria S. Ekman & Svensson for the *M. sylvicola* group (Ekman & Svensson 2014). Species delimitation has perhaps been especially problematic in the *M. prasina* group which includes the type species of the genus, *M. prasina* Fr. (Andersen & Ekman 2005; Sérusiaux *et al.* 2010; Schmull *et al.* 2011). In his European monograph, Coppins (1983) delimited the group based on morphological, anatomical and chemical features: all species have a "micareoid" photobiont (coccoid green alga with cells 4·0–7·5 μm diam.), immarginate apothecia, branched paraphyses and an ascus of the *Micarea*-type (Hafellner 1984). The majority of the species produce the Sedifolia-grey pigment (K+ violet, C+ violet) which is typically present in the apothecia and pycnidia (Coppins 1983; Czarnota & Guzow-Krzemińska 2010). According to Coppins (1983), the group comprised *M. prasina*, *M. hedlundii* Coppins, *M. levicula* (Nyl.) Coppins and with some uncertainty also *M. misella* (Nyl.) Hedl., *M. melanobola* (Nyl.) Coppins and *M. synotheoides* (Nyl.) Coppins. *Micarea prasina* was treated in a wide sense having a variable morphology and including three chemical races. However, *M. prasina* was eventually shown to be non-monophyletic and two distinct lineages were described as new species: *M. subviridescens* (Nyl.) Hedl. and *M. micrococca* (Körb.) Gams ex Coppins (Coppins 2002). Furthermore, *M. xanthonica* Coppins & Tønsberg (Coppins & Tønsberg 2001) and *M. viridileprosa* Coppins & van den Boom (van den Boom & Coppins 2001) were recognized as members of the *M. prasina* group.

Recently, Czarnota & Guzow-Krzemińska (2010) conducted a phylogenetic study, based on mtSSU sequences, to investigate species delimitation in the *M. prasina* group. They concluded that *M. micrococca* includes three distinct lineages and recognized two of them at species level, *M. byssacea* (Th. Fr.) Czarnota *et al.* and *M. micrococca* (Körb.) Gams ex Coppins s.s. A third lineage did not have sufficiently clear morphological, distributional and ecological characters to be recognized as a separate species. The results of Czarnota & Guzow-Krzemińska (2010) show that the variation within the *M. prasina* group, and more specifically in *M. micrococca* and *M. byssacea*, needs to be studied in more detail using information from several gene regions.

According to previous single-gene phylogenetic studies (Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016), *M. byssacea* and *M. micrococca* form a monophyletic species group together with *M. viridileprosa* and the undescribed lineage discovered by Czarnota & Guzow-Krzemińska (2010). In general, *M. byssacea* and *M. micrococca* are characterized by immarginate, convex to hemispherical apothecia and a thallus composed of goniocysts. These species are mostly epiphytes or rarely grow on decaying wood in various woodland habitats. More specifically, the species in the *M. byssacea* and *M. micrococca* complexes differ from each other in the size of apothecia: species in the *M. byssacea* complex form larger apothecia (0·3–0·6 mm diam.) than the species in the *M. micrococca* complex (0·2–0·4 mm diam.).

In the present study, the species diversity within the *M. byssacea* and *M. micrococca* species complexes is further investigated. We use phenotypic characters and multiloci sequence data (ITS, mtSSU and *Mcm7*) to examine the phylogenetic relationships and species delimitation in the two species complexes. Due to the relatively few distinct phenotypic traits, we decided to search for new characters for species delimitation. Crystalline granules in sections of apothecia and thalli, examined in polarized light, have been used in the identification of crustose lichen species in genera such as *Lecanora* and *Mycobilimbia* (Brodo 1984; Spribille *et al.* 2011). In these genera, the presence, distribution, size and solubility of the granules are considered important features. However, their significance in many lichen groups, including *Micarea*, is still poorly known (Orange *et al.* 2010).

Material and Methods

Twenty-five taxa corresponding to the *Micarea prasina* group (*sensu* Andersen 2004; Czarnota & Guzow-Krzemińska 2010; Sérusiaux *et al.* 2010) were used in this study. It is based on material collected from Finland, the Netherlands, Poland, Sweden, Scotland and the USA during 2002–2015. Type material of related *Micarea*

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

TABLE 1. *Specimens of* Micarea *used in the phylogenetic analyses. New species and new sequences generated for the current study are in bold.*

Specimen	Country	Collector, collection number, DNA sample number (where appropriate), herbarium	GenBank Accession number		
			ITS	mtSSU	*Mcm7*
Micarea peliocarpa	USA	*Launis* 66123, DNA A324, (H)	**MG521544**	**MG707741**	**MG692505**
M. adnata	Norway	*Andersen* 48 (BG)	—	AY567751	—
M. byssacea	Finland	*Launis* 289103, DNA A98, (H)	**MG521562**	**MG707768**	**MG692527**
M. byssacea	Finland	*Launis* 289102, DNA A97, (H)	**MG521563**	**MG707769**	**MG692528**
M. byssacea	Finland	*Launis* 289101, DNA A96, (H)	**MG521564**	**MG707770**	**MG692529**
M. czarnotae	Poland	*Czarnota* 3632 (GPN)	—	EF453668	—
M. czarnotae	Poland	*Czarnota* 4179 (GPN)	—	EF453691	—
M. czarnotae	Poland	*Czarnota* 3179 (GPN)	—	EF453674	—
M. czarnotae	Poland	*Czarnota* 4059 (GPN)	—	EF453663	—
M. czarnotae	Finland	*Launis* 109111, DNA A604, (H)	—	**MG707759**	—
M. czarnotae	Finland	*Launis* 1010133, DNA A455, (H)	**MG521557**	**MG707760**	**MG692517**
M. czarnotae	Belgium	*P. van den Boom* 50312, DNA 3712, (LG)	—	**MG707761**	—
M. elachista	Finland	*Launis* 67113, DNA A340, (H)	**MG521548**	**MG707745**	—
M. globulosella	Finland	*Launis* 67112, DNA A240, (H)	**MG521546**	**MG707743**	**MG692507**
M. globulosella	Finland	*Launis* 67114, DNA A243, (H)	**MG521547**	**MG707744**	**MG692508**
M. hedlundii	Finland	*Launis* 67119, DNA A254, (H)	**MG521551**	**MG707749**	**MG692512**
M. herbarum	Netherlands	*Brand* 63193 (LG)	—	KX459350	—
M. herbarum	Netherlands	*P. & G. van den Boom* 52575 (LG)	—	KX459349	**MG692513**
M. laeta	Finland	*Launis* 59153, DNA A825, (H)	**MG521565**	**MG707771**	**MG692530**
M. laeta	Finland	*Launis* 49151, DNA A819, (H)	**MG521566**	**MG707772**	**MG692531**
M. laeta	Finland	*Launis* 59154, DNA A824, (H)	**MG521567**	**MG707773**	**MG692532**
M. laeta	Finland	*Launis* 59155, DNA A827, (H)	—	**MG707774**	**MG692533**
M. laeta	Finland	*Launis* 49152, DNA A823, (H)	—	**MG707775**	**MG692534**
M. laeta	Finland	*Launis* 186152, DNA A803, (H)	—	—	**MG692535**
M. laeta	Finland	*Launis* 269141, DNA A806, (H)	—	**MG707776**	**MG692536**
M. laeta	Finland	*Launis* 286151, DNA A816, (H)	—	**MG707777**	**MG692537**
M. laeta	Finland	*Launis* 1010133, DNA A477, (H)	**MG521568**	**MG707778**	**MG692538**
M. laeta	Finland	*Launis* 1010134, DNA A478, (H)	**MG521569**	**MG707779**	**MG692539**
M. laeta	Finland	*Launis* 1510131, DNA A762, (H)	—	**MG707780**	**MG692540**
M. laeta	Finland	*Launis* 1010135, DNA A427, (H)	**MG521570**	**MG707781**	**MG692541**
M. microareolata	Sweden	*Launis* 148131, DNA A393, (H)	**MG521558**	**MG707762**	**MG692518**

TABLE 1. (*continued*).

Specimen	Country	Collector, collection number, DNA sample number (where appropriate), herbarium	GenBank Accession number		
			ITS	mtSSU	*Mcm7*
M. microareolata	Sweden	*Launis* 148132, DNA A394, (H)	**MG521559**	**MG707763**	**MG692519**
M. microareolata	Finland	*Launis* 59152, DNA A826, (H)	**MG521560**	**MG707764**	**MG692520**
M. microareolata	Finland	*Pykälä* 47783, DNA A798, (H)	—	—	**MG692521**
M. microareolata	Finland	*Pykälä* 47787, DNA A797, (H)	—	**MG707765**	**MG692522**
M. microareolata	Finland	*Launis* 59133, DNA A565, (H)	**MG521561**	**MG707766**	**MG692523**
M. microareolata	Finland	*Launis* 89133, DNA A629, (H)	—	**MG707767**	**MG692524**
M. microareolata	Finland	*Launis* 186151, DNA A802, (H)	—	—	**MG692525**
M. microareolata	Finland	*Pykälä* 47948, DNA A801, (H)	—	—	**MG692526**
M. micrococca	Finland	*Launis* 299101, DNA A100, (H)	MG521552	MG707753	MG692514
M. micrococca	USA	*Launis* 146127, DNA A320, (H)	MG521553	MG707754	MG692515
M. misella	Finland	*Launis* 108111, DNA A264, (H)	MG521545	MG707742	**MG692506**
M. nowakii	Finland	*Launis* 245131, DNA A684, (H)	—	MG707751	—
M. nowakii	Poland	*Czarnota & Guzow-Krzemińska* 4181 (GPN)	—	EF453688	—
M. prasina	Finland	*Launis* 265101, DNA A92, (H)	MG521549	MG707747	MG692510
M. prasina	Finland	*Launis* 199105, DNA A93, (H)	MG521550	MG707748	MG692511
M. prasina	USA	*Tønsberg* 30856 (BG)	—	AY756452	—
M. pseudomicrococca	Finland	*Launis* 59151, DNA A811, (H)	**MG521554**	**MG707755**	—
M. pseudomicrococca	Finland	*Launis* 89132, DNA A599, (H)	**MG521555**	**MG707756**	—
M. pseudomicrococca	Finland	*Launis* 258131, DNA A603, (H)	—	**MG707757**	—
M. pseudomicrococca	Scotland	*Launis* 171141, DNA A645, (H)	**MG521556**	**MG707758**	**MG692516**
M. pycnidiophora	USA	*Tønsberg* 30881 (BG)	—	AY567754	—
M. soralifera	Poland	*Kukwa* 13001 (GPN)	KT119887	KT119886	—
M. soralifera	Finland	*Launis* 1710131, DNA A714, (H)	—	MG707746	MG692509
Micarea sp. lineage A	Scotland	*Launis* 171142, DNA A648, (H)	**MG521571**	**MG707782**	**MG692542**
M. stipitata	USA	*Ekman* s. n.	—	AY567753	—
M. subviridescens	Scotland	*Czarnota* 3599 (GPN)	—	EF453666	—
M. synotheoides	Norway	*Andersen* 47 (BG)	—	AY567756	—
M. tomentosa	Finland	*Launis* 11013, DNA A773, (H)	—	MG707750	—
M. tomentosa	Poland	*Czarnota* 3949 (GPN)	—	EF453686	—
M. viridileprosa	Poland	*Czarnota* 3436 (GPN)	—	EF453671	—
M. viridileprosa	Poland	*Czarnota* 3869 (GPN)	—	EF453673	—
M. xanthonica	USA	*Tønsberg* 25674 (BG)	—	AY756454	—

species from the herbaria G, H, and UPS was studied for comparison, and the type specimens placed under synonymy of *M. micrococca* by Czarnota (2007) were also examined. Detailed information of the material used in the phylogenetic analyses is presented in Table 1.

DNA extraction and sequencing

DNA was extracted from apothecia of specimens which were a maximum of three years old ($n = 1–3$). For most specimens, DNA was extracted using DNeasy® Blood & Tissue kit by Qiagen following the protocol described in Myllys *et al.* (2011). PCR reactions were prepared using PuReTaq Ready-To-Go PCR beads (GE Healthcare). The 25 µl reaction volume contained 19 µl of dH_2O, 1 µl of each primer (10 µM) and 4 µl of extracted DNA.

For the ITS region, PCR was run under the following conditions: initial denaturation for 5 min at 95 °C followed by five cycles of 30 s at 95 °C (denaturation), 30 s at 58 °C (annealing) and 1 min at 72 °C (extension); in the remaining 40 cycles the annealing temperature was decreased to 56 °C, the PCR program ended with a final extension for 7 min at 72 °C. The primers ITS1-LM (Myllys *et al.* 1999) and ITS4 (White *et al.* 1990) were used both for PCR amplification and for sequencing of the nuclear ribosomal ITS region.

For the mtSSU region, PCR was run under the following conditions: initial denaturation for 10 min at 95 °C followed by six cycles of 1 min at 95 °C (denaturation), 1 min at 62 °C (annealing) and 105 s at 72 °C (extension); in the remaining 35 cycles the annealing temperature was decreased to 56 °C and the extension time to 1 min, the PCR program ended with a final extension for 10 min at 72 °C. The primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999) were used for both PCR amplification and sequencing.

For the *Mcm7* region, PCR was run under two different conditions depending on the primers selected: initial denaturation for 10 min at 94 °C followed by 38 cycles of 45 s at 94 °C (denaturation), 50 s at 55/56 °C (annealing) and 1 min at 72 °C (extension); the PCR program ended with a final extension for 5 min at 72 °C. The primers x. *Mcm7*.f (Leavitt *et al.* 2011) and *Mcm7*.1348R (Schmitt *et al.* 2009) or newly generated primers *Mcm7*_AL1r (5' CKGTCACARCSAAGCARTAYACACCTATG 3') and *Mcm7*_AL2f (5' CTTTYGTCACWCCSCCRATKAGRAGC 3') were used for both PCR amplification and sequencing. The annealing temperature was 56 °C for the first primer pair and 55 °C for the second newly generated primer pair. PCR products were cleaned and sequenced by Macrogen Inc., South Korea (www.macrogen.com).

Phylogenetic analyses

For the analysis 107 sequences were generated and 19 were obtained from GenBank. *Micarea peliocarpa* (Anzi) Coppins & R. Sant. was used as an outgroup for the *M. prasina* group.

A total of 29 ITS sequences, 59 mtSSU sequences and 38 *Mcm7* sequences were aligned separately with MUSCLE v.3.8.31 (Edgar 2004) using EMBL-EBI's freely available web service (http://www.ebi.ac.uk/Tools/msa/muscle/). The single-gene trees did not show any strongly supported conflicts according to the method of Kauff & Lutzoni (2002) (≥75% bootstrap values) and the three matrices were combined into a concatenated matrix in MacClade 4.08 (Maddison & Maddison 2005). Portions of the alignment with ambiguous positions that might not have been homologous were excluded. The concatenated data set, including 63 terminals, was subjected to maximum parsimony analysis as implemented in TNT v.1.1 (Goloboff *et al.* 2008) and to maximum likelihood analysis using RAxML v.8.1.15 (Stamatakis 2014). The parsimony analysis was performed using the "Traditional Search" with random addition of sequences with 100 replicates and the tree bisection and reconnection (TBR) branch-swapping algorithm. Ten trees were saved for each replicate and gaps were treated as missing data. Node support was estimated using bootstrapping with 1000 replicates. Bootstrap values >75% are considered significant. For the maximum likelihood analysis, the combined data set was assigned to seven partitions: ITS1, 5.8S, ITS2, mtSSU and three codon positions of *Mcm7*. The hypervariable region near the end of the mtSSU was removed from the analyses (characters 649–804 in the alignment). We used an independent GTR + G model for each subset and branch lengths were assumed proportional across subsets. The tree with the highest likelihood from 36 individual runs was selected. Node support was estimated with 1000 bootstrap replicates using the rapid bootstrap algorithm.

Morphology and chemistry

Hand-cut apothecial sections and squashed thallus preparations were examined with a dissecting or compound microscope. Ascospore dimensions and other anatomical measurements were made on material mounted in water. Chemical spot tests were performed under a compound microscope using sodium hypochlorite (C) and 10% potassium hydroxide (K) (Orange *et al.* 2010). Pigments were characterized following Coppins (1983), Meyer & Printzen (2000) and Czarnota (2007). Specimens were further studied using thin-layer chromatography (solvent C) following Culberson & Kristinsson (1970) and Orange *et al.* (2010), and crystalline granules were examined using a compound microscope with polarized light. The crystalline granules were studied from sequenced specimens within the *M. micrococca* and *M. byssacea* complexes, and from specimens of *M. prasina*. Specimens are deposited in BG, GPN, H, LG and E.

Results

In this study a total of 107 new sequences were generated and 19 sequences were downloaded from GenBank. The final 3-loci data set consisted of 126 sequences and 1825

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

characters, of which 720 were parsimony-informative. Since the topologies of the maximum likelihood and TNT analyses did not show any strongly supported conflicts, only the tree obtained from the maximum likelihood analysis is shown (Fig. 1).

Our multiloci phylogeny agrees with the previous single-locus phylogenies for this group (Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016) and shows that the *Micarea prasina* group is strongly supported and monophyletic.

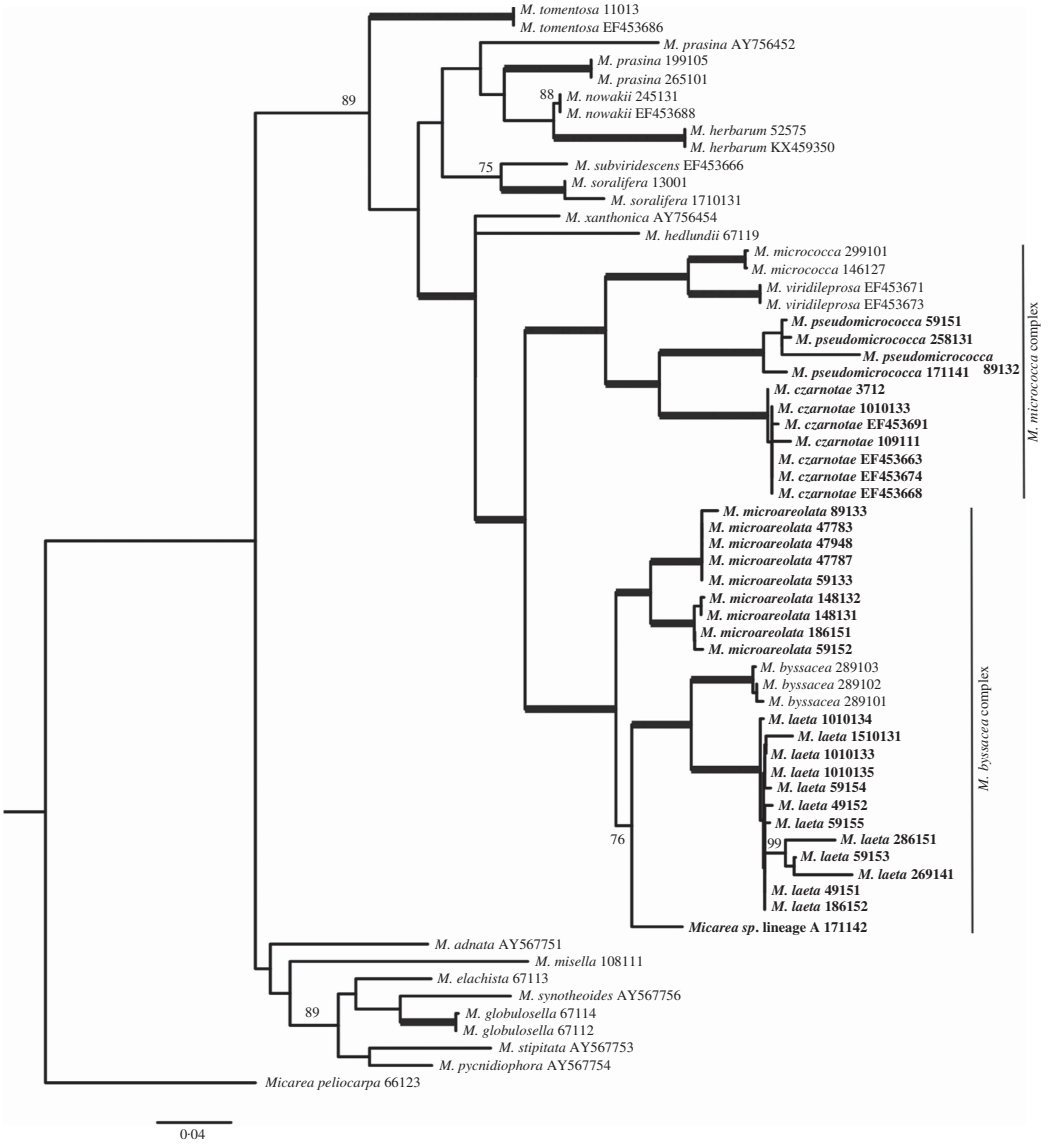

FIG. 1. Phylogenetic relationships of *Micarea czarnotae* sp. nov., *M. laeta* sp. nov., *M. microareolata* sp. nov., *M. pseudomicrococca* sp. nov. and *Micarea* sp. lineage A (all shown in bold) as revealed by a maximum likelihood phylogram generated from RAxML analysis based on the combined ITS, mtSSU and *Mcm7* data set. Bootstrap values ≥75% in both analyses (RAxML and TNT) are indicated by thickened branches. Branches which are supported solely by maximum likelihood analysis bootstrap values ≥75% are shown above nodes.

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

Furthermore, *M. byssacea* and *M. micrococca* are sister groups and form strongly-supported monophyletic species complexes with five previously undescribed new lineages.

The *M. byssacea* complex is divided into four lineages: 1) *M. microareolata*, represented by nine specimens in our study; 2) a single unidentified individual (lineage A in Figs 1 & 4D) collected from Scotland; 3) *M. byssacea* s.s., with three specimens and 4) *M. laeta*, represented by 12 specimens. *Micarea byssacea* s.s. and *M. laeta* form a strongly supported sister group. Additionally, a cryptic lineage is quite likely present within *M. microareolata*.

The *M. micrococca* complex consists of four distinct well-supported groups. The two clades, *M. viridileprosa* and *M. micrococca* s.s. (Czarnota & Guzow-Krzemińska 2010), form a strongly supported sister group. The remaining two clades represent new species: *M. czarnotae* with seven specimens (corresponding to *M. micrococca* "B" in Czarnota & Guzow-Krzemińska (2010)) and *M. pseudomicrococca*, represented by four specimens in our phylogeny.

Small crystalline granules, soluble in K, were detected in polarized light in all species studied (Fig. 2). Such granules were present in both the hymenium and thallus of *M. byssacea*, *M. laeta*, *M. microareolata*, *M. pseudomicrococca* and *M. micrococca*. In contrast to other species, *M. czarnotae* formed granules only in the hymenium and never in the thallus. Crystalline granules were also investigated in *M. prasina* s.s. (Fig. 4C) because of its morphological resemblance to species in the *M. byssacea* and *M. micrococca* complexes. *Micarea prasina* formed crystals in the epihymenium and the thallus (Fig. 2D) but, unlike the other species, never in the hymenium (*M. prasina* sample AY756452, resolved as a different branch in the analyses, but was not studied). Without exception, identical crystalline features were present in all individuals within each of the species studied. It should be noted that crystalline granules were examined only in sequenced specimens, as this is the most reliable way of species identification. Consequently, the number of specimens studied was limited.

Discussion

Our multiloci phylogeny corresponds well with the previous single-locus phylogenies of the *M. prasina* group (Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016; van den Boom *et al.* 2017). Generally, the clades were strongly supported despite the rather large quantity of missing data, especially in the ITS regions (see Table 1). Based on predominantly new collections, the present study revealed five previously undescribed, well-supported lineages. These lineages are also supported by morphological traits. Four of the lineages represent new species, for which the following names are proposed: *Micarea pseudomicrococca* Launis & Myllys, *Micarea czarnotae* Launis, van den Boom, Sérusiaux & Myllys, *Micarea microareolata* Launis, Pykälä & Myllys and *Micarea laeta* Launis & Myllys.

The fifth previously undescribed lineage is represented by only a single sample collected from decaying wood in eastern Scotland. This putative new taxon forms pallid, 0·2–0·7 mm diam. apothecia, resembling in size and shape those of *M. byssacea,* except that they always lack the Sedifolia-grey pigment (K− and C−). Furthermore, this new taxon forms a bright green thallus composed of goniocysts strongly resembling the thallus of *M. micrococca*. Owing to insufficient material, no taxonomic innovation is proposed at this time. However, this result indicates that there is insufficient knowledge of the diversity within the *M. prasina* group, and more precisely within the *M. byssacea* complex, even in well-studied areas of Europe.

Two of the new species, *M. czarnotae* and *M. pseudomicrococca*, belong to the *M. micrococca* complex while *M. microareolata* is part of the *M. byssacea* complex. Our results show that species in the two groups differ mainly in the size and shape of the apothecia. Species in the *M. micrococca* complex, including the new species described in this study, have small apothecia that are plane, convex, hemispherical or sometimes tuberculate and 0·2–0·4 mm diam. Species in the *M. byssacea* complex are characterized by wider apothecia that are 0·3–0·6

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

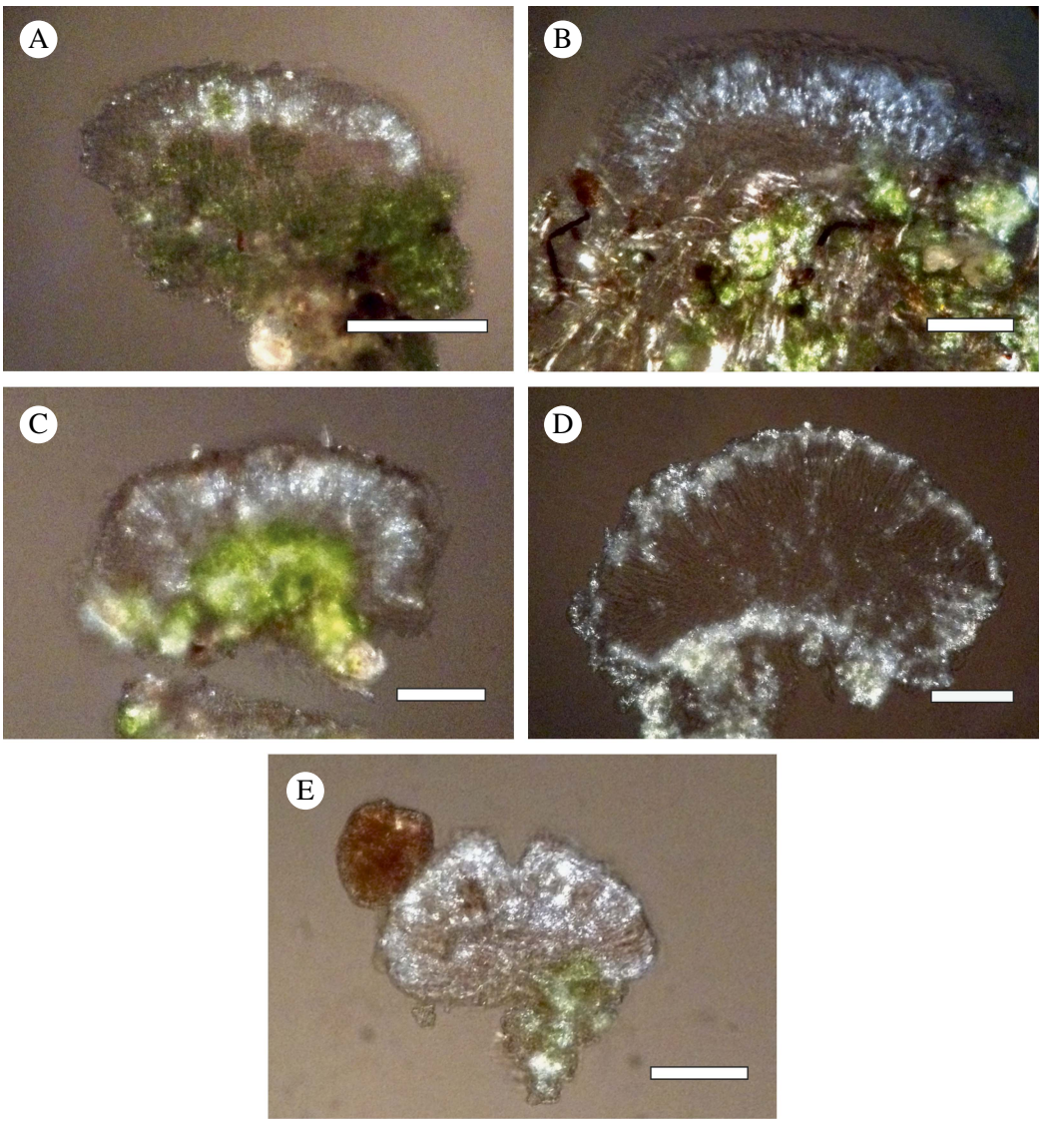

FIG. 2. Crystalline granules in apothecial sections, detected in polarized light. A, *Micarea czarnotae* (holotype); B, *M. laeta* (holotype); C, *M. microareolata* (holotype); D, *M. prasina* (*Launis* 229106, H); E, *M. pseudomicrococca* (holotype). Scale bars = 100 μm. In colour online.

(–0·7) mm diam., adnate, convex to hemispherical or sometimes tuberculate. Results based on molecular data show that these subtle phenotypic differences are significant in defining species boundaries in the *M. byssacea* and *M. micrococca* complexes.

Micarea czarnotae produces the Sedifolia-grey pigment (K+ violet and C+ violet), whereas *M. micrococca* and *M. pseudomicrococca* do not. Furthermore, thallus morphology and colour differ between the species: *M. micrococca* has a bright green or olive green thallus composed of coalescing granules, whereas *M. pseudomicrococca* has an olive green, minutely granular thallus and *M. czarnotae* an olive green, densely granular, warted-areolate or,

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

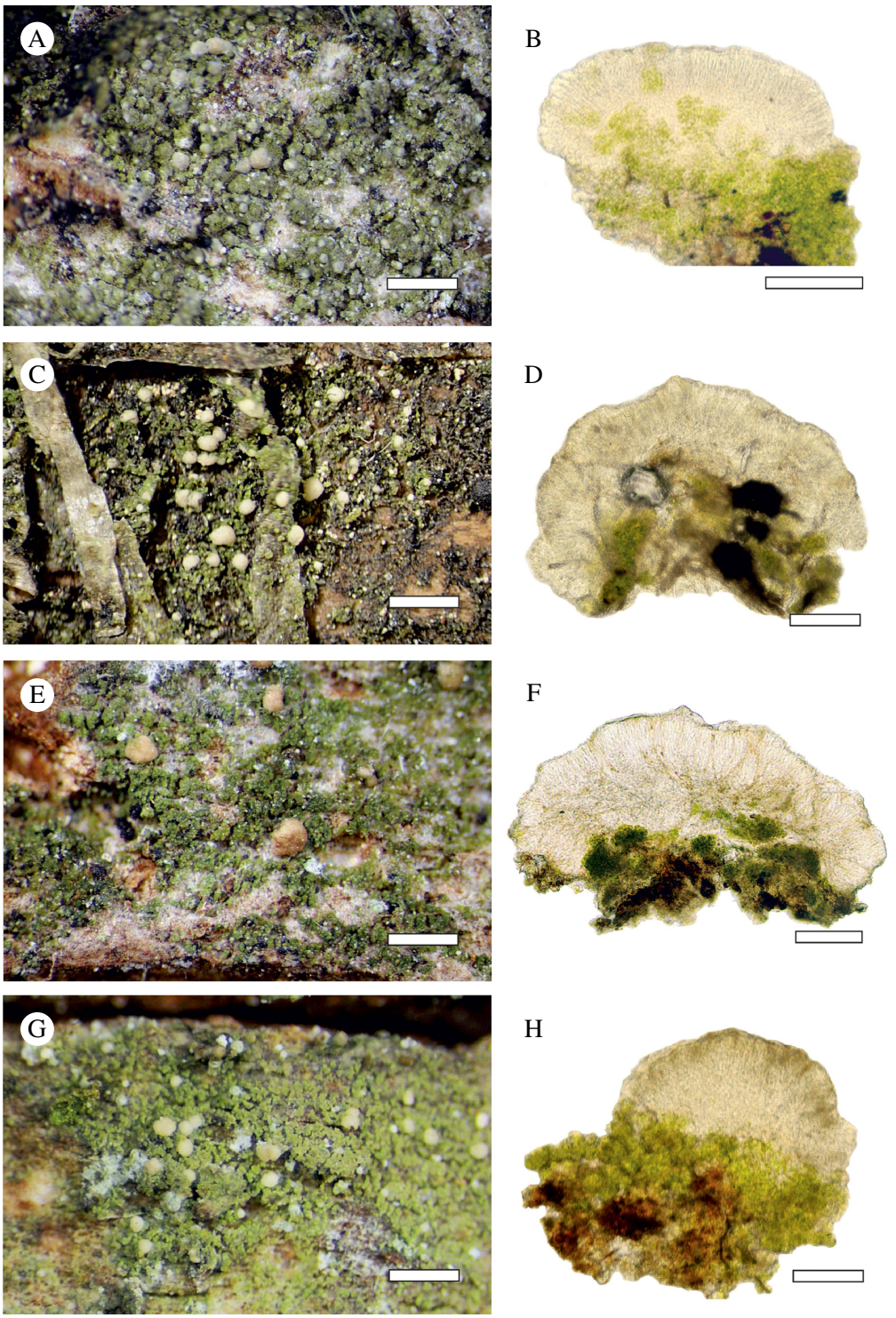

FIG. 3. A & B, *Micarea czarnotae* (holotype); A, habitus; B, apothecial section. C & D, *M. laeta* (holotype); C, habitus; D, apothecial section. E & F, *M. microareolata* (*Pykälä* 47787, H); E, habitus; F, apothecial section. G & H, *M. pseudomicrococca* (holotype); G, habitus; H, apothecial section. Scale bars: A, C, E & G = 1 mm; B, D, F & H = 100 μm. In colour online.

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

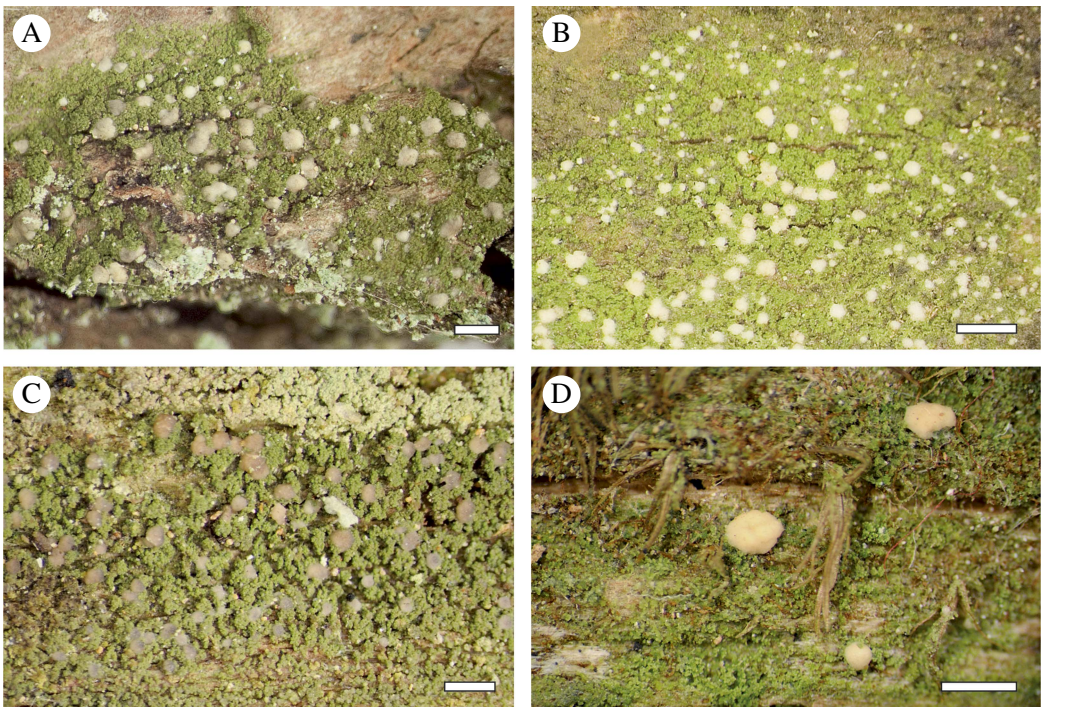

FIG. 4. A, *Micarea byssacea* (*Launis* 66128, H) habitus; B, *M. micrococca* s.s. (*Launis* 1010131, H) habitus; C, *M. prasina* (*Launis* 229106, H) habitus; D, *Micarea* sp. lineage A (*Launis* 171142, H, see Fig. 1) habitus. Scale bars = 1 mm. In colour online.

when well developed, an almost continuous and cracked thallus.

Micarea byssacea produces the Sedifolia-grey pigment (K+ violet and C+ violet), whereas *M. laeta* and *M. microareolata* do not. Furthermore, thallus colour and morphology differ between the species in the *M. byssacea* complex: *M. byssacea* is usually characterized by an olive green, minutely granular thallus, *M. microareolata* by a whitish or pale olive green thallus composed of small areolae, and *M. laeta* by a vivid green or olivaceous thallus composed of coalescing granules.

Finding appropriate morphological and chemical characters is one of the major challenges in species delimitation of lichen-forming fungi, especially in groups where characters are few or highly homoplastic (see Lumbsch & Leavitt 2011; Mark *et al.* 2016). Crystalline granules have not previously been examined in the genus *Micarea* and thus their value in the identification of *Micarea* spp. was unknown. Our study shows that crystalline features are, at least in some cases, useful as a species-level character. The presence and distribution of such granules were found to be unique in *M. prasina* (granules only in the epihymenium, Fig. 2D) and in *M. czarnotae* (no crystalline granules in the thallus, Fig. 2A). Within the *M. byssacea* complex, crystalline features were not found to be useful because the size and distribution of these granules were shown to be identical amongst the species. Many of the crystalline deposits found in lichens are composed of calcium oxalate (Orange *et al.* 2010) but the detailed composition of the crystalline granules detected in the *M. prasina* group is unknown. The presence, distribution and quantity of crystals were shown to be unaffected by light conditions, apothecial pigments and other anatomical or environmental features. The crystalline granules were studied in

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

sequenced specimens as this was the most reliable way to delimit species in this phenotypically challenging group. However, this restriction limited the number of specimens used to investigate for the presence of crystalline granules. Therefore, to truly understand the reliability of the new feature as a species-level character within *Micarea*, a larger data set is needed. Several taxonomic problems in the *M. prasina* group still remain to be addressed. In light of this study, and those of Czarnota (2007), Czarnota & Guzow-Krzemińska (2010), Brand *et al.* (2014), Guzow-Kremińska *et al.* (2016) and van den Boom *et al.* (2017), some of the type specimens synonymized with *M. prasina* Fr. (e.g. *M. melanobola* (Nyl.) Coppins) should be investigated in more detail. Additionally, the infraspecific genetic variation between European and American specimens of *M. prasina* s.s. should be examined. These questions are currently under consideration and are expected to be addressed in the near future.

Key to the *Micarea byssacea* and *M. micrococca* complexes in Europe

1 Thallus containing methoxymicareic acid, apothecia usually present and abundant 2
Thallus and apothecia containing gyrophoric acid (C+ red), apothecia usually absent or rarely few. 7

2(1) Apothecia up to 0·6(–0·7) mm diam., often adnate (*M. byssacea* complex) 3
Apothecia up to 0·4 mm diam., rarely adnate (*M. micrococca* complex) 5

3(2) Thallus minutely granular, olive green, apothecia usually greyish (K+ and C+ violet) . . . . . **M. byssacea**
Thallus granular or areolate, vivid green, olive green, pale olive green, whitish green or sometimes partly bright green, apothecia whitish to brownish (K− and C−) 4

4(3) Thallus usually areolate, apothecia cream-white, ascospores 2·2–3·0 µm wide . . . . . **M. microareolata**
Thallus granular and/or continuous, apothecia cream-white or brownish, ascospores 3–4 µm wide . . . . . **M. laeta**

5(2) Thallus granular, bright green, apothecia whitish, ascospores 3·0–4·5 µm wide . . . . . **M. micrococca**
Thallus olive green, granular and/or continuous crust, ascospores 2·0–3·2(–3·5) µm wide 6

6(5) Thallus warted-areolate, cracked to continuous without crystalline granules, apothecia greyish tinged (K+ and C+ violet), paraphyses up to 1·5 µm wide . . . . . **M. czarnotae**
Thallus granular with crystalline granules visible in polarized light, apothecia whitish cream (K− and C−), two types of paraphyses, up to 2·0 µm wide . . . . . **M. pseudomicrococca**

7(1) Thallus ± leprose, bright green . . . . . **M. viridileprosa**

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press

The Species

For descriptions of *M. byssacea* and *M. micrococca*, see Czarnota & Guzow-Krzemińska (2010). Even with the recognition of *M. laeta*, the description of *M. byssacea* is still valid but specimens of *M. byssacea* with completely pallid apothecia should be examined carefully. We studied all synonyms placed under *M. byssacea* and *M. micrococca* (Czarnota 2007) with relevant conclusions presented below species descriptions.

The mtSSU sequences of *M. byssacea* and *M. micrococca* s.s. used in the phylogenetic analysis (Fig. 1) are identical to those used and identified by Czarnota & Guzow-Krzemińska (2010).

Micarea czarnotae Launis, van den Boom, Sérusiaux & Myllys sp. nov.

MycoBank No.: MB 824291

Thallus olive green to darkish olive green, goniocysts often coalescing to form dense ± continuous thallus, sometimes cracked, if less developed warted-areolate; apothecia numerous, crowded, up to 0·3 mm diam., cream-white or pale brownish, often greyish tinge (K± violet, C± violet); ascospores oblong-ellipsoid or obovoid, 0–1 septate, 7·0–10·0 × 2·25–3·5 µm; production of methoxymicareic acid. Resembles *M. micrococca* and *M. pseudomicrococca* but differs by having variously coloured apothecia and by producing the Sedifolia-grey pigment. In addition, *M. czarnotae* lacks crystalline granules in the thallus.

Type: Finland, Varsinais-Suomi, Nummi-Pusula, Myllypuro, mixed forest between Vahermanjärvi and Tarkeelanjärvi, near River Myllypuro, on bark of *Pinus sylvestris*, in N-facing shaded and moist microhabitat, YKJ N6719586, E3335308, 2011, *Launis* 109111 (H—holotype). GenBank Accession numbers: ITS, MG521557; MtSSU, MG707759, MG707760, MG707761; *Mcm7*, MG692517.

(Fig. 3A & B)

Thallus effuse, olive green to darkish olive green, usually ± thick, granular, composed of goniocysts 20–35(–40) µm diam.; goniocysts usually coalescing to form a dense almost continuous thallus, sometimes cracked, if less developed warted-areolate. *Photobiont* micareoid, algal cells 4·5–7·5 µm diam.

Apothecia numerous, often crowded, small, 0·1–0·3 mm, usually plane or hemispherical, sometimes becoming tuberculate (and then up to 0·4 mm diam.), cream-white or brownish, often with a greyish tinge due to the Sedifolia-grey pigment (K± violet and C± violet). *Hypothecium* hyaline. *Hymenium* hyaline, *c.* 30–45 µm high. *Epihymenium* hyaline or pale grey, K± violet and C± violet. *Paraphyses* numerous, branched, 1·0–1·5 µm wide, apices not wider. *Asci* clavate, *Micarea*-type, 35–40 × 8–10 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1 septate, 7·0–10·0 × 2·25–3·5 µm.

Pycnidia of two types, whitish, usually K− and C−, sometimes K± violet and C± violet (Sedifolia-grey pigment). *Mesopycnidia* often numerous and sessile, sometimes immersed in surrounding goniocysts, *c.* 70–100 µm wide, globose or doliform, sometimes with gaping ostiole extruding white conidial mass. *Mesoconidia* cylindrical or cylindrical-fusiform, 4·0–5·0(–5·5) × 1·0–1·5 µm. *Micropycnidia* immersed in surrounding goniocysts or sessile, 80–130 µm wide, globose, if sessile often with gaping ostiole. *Microconidia* filiform to narrowly fusiform, 5·5–7·0 × 0·8–1·0(–1·2) µm.

Crystals (studied in polarized light). Visible in hymenium, none detected in the thallus. Soluble in K (Fig. 2A).

Chemistry. Methoxymicareic acid.

Etymology. The species is named after our colleague Dr Pawel Czarnota for his significant contribution to the study of the genus *Micarea*, and for collecting the first known specimens of *M. czarnotae*.

Habitat and distribution. Known from bark of *Pinus sylvestris*, wood and bark of *Picea abies*, bark of *Quercus* sp. and twigs of *Alnus glutinosa*. Several specimens were collected from humid environments near a bog or river, or from standing tree trunks on the northern side or from near the ground. *Micarea czarnotae* is so far known from Southern Finland, Poland and the Netherlands.

Notes. Micarea czarnotae was first introduced by Czarnota & Guzow-Krzemińska (2010) as "*M. micrococca* B", a transitional morphotype between *M. micrococca* and *M. byssacea*. Because of the lack of clear

morphological, distributional, ecological and, above all, molecular multiloci data, no taxonomic innovations were proposed at that time. Our study, however, shows that *M. czarnotae* is both molecularly and morphologically a distinct species-level taxon.

Micarea czarnotae forms small, convex to hemispherical apothecia resembling those of *M. micrococca* and *M. pseudomicrococca*. However, its apothecia are often variously coloured and K± violet, C± violet when the Sedifolia-grey pigment is present. It also differs from *M. micrococca* and *M. pseudomicrococca* in characters detectable in polarized light: *M. czarnotae* does not produce crystalline granules in its thallus whereas *M. micrococca* and *M. pseudomicrococca* always do.

Micarea byssacea differs in larger, often adnate apothecia and a minutely granular thallus that is never densely continuous or cracked (Fig. 4A). In addition, *M. byssacea* produces crystalline granules in the thallus and a hymenium detectable in polarized light.

Additional specimens examined. **Finland:** *Uusimaa*: Tuusula, near Korso, *Picea abies*-dominated managed forest, shaded and dense, on wood of fallen, decaying (late-stage) *Picea abies*, ETRS-TM35FIN N 6692506, E 391428, 2013, *Launis* 1010133 (H).—**The Netherlands:** *Noord-Brabant*: W of Son, S of Bestseweg, 51° 30'39"N, 5°27'41"E, 30 m alt., small *Pinus* forest, on fallen rotting trunk, 2014, *P. & B. van den Boom* 50312 (LG, hb v.d. Boom).—**Poland:** *Kotlina Sandomierska*: Płaskowyż Kolbuszowski, *c.* 2 km SE of Wilcza Wola Village, 50°19'69"N, 21°58'23"E, *c.* 120 m. alt., on bark of *Pinus sylvestris* within wet pine forest, 2003, *Czarnota* 3632 (GPN). *Wzniesienia Łódzkie*: Wzniesienia Łódzkie Landscape Park, Tadzin forest district, forest section no. 110, *c.* 1 km W of Tadzin Village, 51°49'39"N, 19° 44'33"E, *c.* 190 m alt., on bark of *Quercus* sp. within mixed pine-oak forest, 2004, *Czarnota* 4179 (GPN). *Pojezierze Chełmińsko-Dobrzyńskie, Garb Lubawski*: Park Krajobrazowy Wzgórz Dylewskich, oddz. 97c., on twigs of *Alnus glutinosa* within alder bog forest (no coordinates available), 2002, *Czarnota* 3179 (GPN) & *Kukwa*. *Beskid Niski Mts*: SW slope of Piotruś Mt., above Stasianie settlement in valley of Jasiolka River, 49°28'02"N, 21° 44'20"E, *c.* 500 m alt., on bark at the base of *Picea abies* trunk within Carpathian beech forest, 2004, *Czarnota* 4059 (GPN).

Micarea laeta Launis & Myllys sp. nov.

MycoBank No.: MB 824294

Thallus effuse, vivid green to olive green, composed of goniocysts, granular or almost continuous crust, if less developed small warted or warted-areolate; apothecia numerous, usually cream-white, sometimes brownish, up to 0·5(–0·6) mm, adnate, convex to hemispherical, rarely subglobose, simple or tuberculate; ascospores oblong-ellipsoid or obovoid, 0–1-septate, (8·0–)8·5–12·0 × 3·0–4·0 µm; production of methoxymicareic acid. Resembles *M. byssacea* and *M. microareolata* but differs from *M. byssacea* by lacking the Sedifolia-grey pigment and often forming a more aggregated or continuous thallus. *Micarea microareolata*, in contrast, has narrower ascospores and usually an areolate thallus.

Type: Finland, Etelä-Häme, Jyväskylä, Korpilahti, *Picea abies*-dominated mixed managed forest, on bark of standing decaying *Betula* sp., on shaded N-side of the tree, YKJ E3418597, N6885262, 5 September 2015, *Launis* 59153a (H—holotype), 59153b (E—isotype). GenBank Accession numbers: ITS: MG521565, MG521566, MG521567, MG521568, MG521569, MG521570. MtSSU: MG707771, MG707772, MG707773, MG707774, MG707775, MG707776, MG707777, MG707778, MG707779, MG707780, MG707781. *Mcm7*: MG692530, MG692531, MG692532, MG692533, MG692534, MG692535, MG692536, MG692537, MG692538, MG692539, MG692540, MG692541.

(Fig. 3C & D)

Thallus effuse, vivid green to olive green, usually rather thin, composed of goniocysts 17–40 µm diam.; goniocysts usually coalescing to form larger granules or an almost continuous crust, if less developed small warted or warted-areolate. *Photobiont* micareoid, algal cells 4·5–7·5 µm diam.

Apothecia numerous, whitish or usually creamy-white, sometimes brownish, 0·3–0·5 (–0·6) mm, adnate, convex to hemispherical, rarely subglobose, simple or becoming tuberculate (and then up to 0·6 mm diam.), always K− and C−. *Hypothecium* hyaline. *Hymenium* hyaline *c.* 35–50 µm high. *Epihymenium* hyaline. *Paraphyses* numerous, branched, 1·0–1·5(–1·8) µm wide, apices barely wider. *Asci* clavate, *Micarea*-type, 35–40 × 8–10 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1-septate, (8·0–)8·5–12·0 × 3·0–4·0 µm.

Pycnidia of two types, whitish, K− and C−. *Mesopycnidia* usually numerous, globose or doliform, 40–90 µm wide, usually immersed in surrounding goniocysts, sometimes sessile with gaping ostiole and extruding white conidial mass. *Mesoconidia* cylindrical or cylindrical-fusiform, 4·0–5·5 × 1·2–1·5 µm. *Micropycnidia* immersed in surrounding

goniocysts, inconspicuous, globose, up to 60 µm wide. *Microconidia* filiform to narrowly fusiform, straight or slightly curved, 5·0–7·5 (–8·0) × 0·8–1·0 µm.

Crystals (studied in polarized light). Visible in hymenium and in thallus. Soluble in K (Fig. 2B).

Chemistry. Methoxymicareic acid.

Etymology. The name is derived from Malme's exsiccate specimen *Micarea prasina* Fr. f. *laeta* Th. Fr. The original etymology chosen by Th. Fries refers to the pale apothecia.

Habitat and distribution. Known from bark of *Betula* sp. and bark and wood of *Picea abies*. So far known from several localities in Southern and Central Finland and Sweden. Specimens were collected from managed and old-growth forests.

Notes. Specimens designated as the newly described species *M. laeta* have been collected many times since 1890 and determined as a form level of *M. prasina* (i.e. *M. prasina* f. *laeta* (Th. Fr.) Hedl (= *Catillaria prasina* f. *laeta* Th. Fr.) (Hedlund 1892)) or treated as a synonym of *M. prasina* Fr. (Coppins 1983) and of *M. byssacea* (Czarnota & Guzow-Krzemińska 2010). In light of this, specimens resembling *M. byssacea* with completely pallid apothecia should be investigated carefully.

As *M. laeta* was first known as a form of *M. prasina*, we considered describing a new combination instead of a new species. However, this was not possible because the original name has been shown to be invalid (see Coppins 1983) since the type specimen of *M. prasina* f. *laeta* is the same as that of *M. prasina*. The taxon is found, for example, in Malme's exsiccate specimens and based on phenotypic characters this specimen is identical to the fresh specimens found in our study. Therefore, we propose the name *M. laeta* for the new species.

To the best of our knowledge, the name 'laeta' has previously been used invalidly only in the level of form of *M. prasina*, and never at species level. Our molecular results clearly show that the taxon we have found and linked to Malme's exsiccate specimens is a species-level unit. As the word 'laeta' refers to pale, it is considered very suitable for the new species with pale apothecia.

Micarea laeta is characterized by a granular thallus, pale apothecia and wide spores. The main morphological features separating it from *M. byssacea* and *M. microareolata* include the structure of the thallus, pigmentation in the apothecia and spore width. *Micarea byssacea* usually produces the Sedifolia-grey pigment in the apothecia, except when growing in deep shade. In addition, it forms a minutely granular thallus that rarely coalesces to form larger granules, or a continuous crust. *Micarea microareolata*, in contrast, has narrower spores and an areolate thallus.

Exsiccati. Malme, *Lichenes Suecici Exsiccati*, No 23 (H) [as *Micarea prasina* Fr. f. *laeta* Th. Fr; Sweden, Södermanland, 1890, *O. Malme*]. Magnusson, *Lichenes Selecti Scandinavici Exsiccati*, No 134 (H) [as *Catillaria prasina* (Fr.) Th. Fr. f. *laeta* Th. Fr; Sweden, Västergötland, 1927, *A. H. Magnusson*].

Additional specimens examined. **Finland:** *Etelä-Häme*: Hämeenlinna, Evo, managed mixed forest, on bark of fallen, decaying *Picea abies*, ETRS-TM35FIN N6787475.7690, E399873.8954, 2013, *Launis* 1510131 (H). *Etelä-Häme*: Jyväskylä, Korpilahti, *Picea abies*-dominated mixed managed forest, on bark of standing decaying *Betula* sp., on shaded N-side of the tree, YKJ E3418597, N6885262, 2015, *Launis* 59153 (H); Jyväskylä, Kuusimäki, mixed managed forest, on bark of standing decaying (early stage) *Picea abies*, YKJ E3425022, N6902706, 2015, *Launis* 49151 (H); *ibid.*, *Picea abies*-dominated mixed managed forest, on bark of standing decaying *Picea abies*, YKJ N6867631, E3459820, 2015, *Launis* 59154, 59155 (H); *ibid.*, mixed managed forest, on bark of standing decaying *Betula* sp., in shade near ground, YKJ E3425062, N6902944, 2015, *Launis* 49152 (H); Joutsa, Höystösensuo, *Pinus sylvestris*-dominated mixed managed forest, on bark of standing decaying *Picea abies*, YKJ E3458859, N6968267, 2015, *Launis* 186152 (H); Joutsa, Leivonmäki, mixed managed forest, on bark of standing decaying *Betula* sp., in shade, YKJ E3443740, N6868132, 2014, *Launis* 269141 (H); Äänekoski, mixed managed forest, on bark of standing decaying *Picea abies*, N-side of the tree in shade, YKJ E3427400, N6959860, 2015, *Launis* 286151 (H). *Uusimaa*: Tuusula, near Korso, *Picea abies*-dominated managed forest, shaded and dense, on wood of fallen, decaying (mid-stage) *Picea abies*, ETRS-TM35FIN N6692506, E391428, 2013, *Launis* 1010133, 1010134, 1010135 (H).

Micarea microareolata Launis, Pykälä & Myllys sp. nov.

MycoBank No.: MB 824292

Thallus pale olive green, whitish green or bright green, goniocysts usually coalescing to form convex to subglobose small areolae; apothecia numerous, whitish or cream-white, up to 0·6(–0·7) mm diam., adnate, convex to hemispherical, K− and C−; ascospores oblong-ellipsoid or obovoid, 0–1 septate, 7·5–12·0 × (2·00–)2·2–3·00 µm; production of methoxymicareic acid. Resembles *M. byssacea* and *M. laeta* but differs from *M. byssacea* by lacking the Sedifolia-grey pigment, forming a more aggregated thallus and by the narrower ascospores. *Micarea laeta* also has pale apothecia but its ascospores are wider than those of *M. microareolata*.

Type: Finland, Etelä-Savo, Jyväskylä, Korpilahti, *Picea abies*-dominated mixed managed forest, on bark of standing decaying *Picea abies*, YKJ E3418403, N6885234, 2015, *Launis* 59152 (H—holotype). GenBank Accession numbers: ITS: MG521558, MG521559, MG521560, MG521561. MtSSU: MG707762, MG707763, MG707764, MG707765, MG707766, MG707767. *Mcm7*: MG692518, MG692519, MG692520, MG692521, MG692522, MG692523, MG692524, MG692525, MG692526.

(Fig. 3E & F)

Thallus effuse, pale olive green, whitish green or sometimes partly bright green, usually rather thin, composed of goniocysts 18–40 µm diam.; goniocysts usually coalescing to form convex to subglobose small areolae (in cross-section goniocysts distinctly visible), areolae effuse or concentrated, sometimes thallus granular or, if less developed, small warted. *Photobiont* micareoid, algal cells 4·5–7·5 µm diam.

Apothecia usually numerous, whitish cream, 0·3–0·6(–0·7) mm diam., adnate, convex to hemispherical, sometimes becoming tuberculate, always K− and C−. *Hypothecium* hyaline. *Hymenium* hyaline, *c.* 30–45 µm high. *Epihymenium* hyaline. *Paraphyses* numerous, richly branched, 1·0–1·8(–2·0) µm wide, apices not wider or only slightly. *Asci* clavate, *Micarea*-type, 25–35 × 9–10 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1-septate, 7·5–12·0 × (2·0–)2·2–3·0 µm.

Pycnidia of two types, small and inconspicuous, whitish, K− and C−. *Mesopycnidia* usually present, immersed in surrounding goniocysts, up to 70 µm wide, sometimes sessile with gaping ostiole extruding white conidial mass. *Mesoconidia* cylindrical or cylindrical-fusiform, 4·0–5·5(–6·0) × 1·0–1·2 (–1·5) µm. *Micropycnidia* immersed in surrounding goniocysts, globose, up to 60 µm wide. *Microconidia* filiform to narrowly fusiform, straight or slightly curved, 5·0–7·5 × 0·8–1·0 µm.

Crystals (studied in polarized light). Visible in hymenium and in thallus. Soluble in K (Fig. 2C).

Chemistry. Methoxymicareic acid.

Etymology. The name *M. microareolata* refers to the areolate morphology of the thallus.

Habitat and distribution. Micarea microareolata is known from bark of *Alnus glutinosa*, *Betula* sp., *Picea abies*, *Salix pentandra* and *Quercus robur* from Southern and Central Finland and southern Sweden. This species seems to have rather broad habitat requirements. Specimens have been collected from well-lit to shaded and from mesic to wet, managed and old-growth forests.

Notes. Micarea microareolata is characterized by a ± pale green areolate thallus, composed of goniocysts, and cream-white apothecia that lack the Sedifolia-grey pigment. In many respects it resembles *M. byssacea* and *M. laeta*, with which it forms a closely related species group. These three species are characterized by similar ecological preferences, and shape and size of the apothecia. In addition, all three species produce methoxymicareic acid and crystalline granules in the apothecia and thallus.

The main morphological features separating *M. microareolata* from *M. byssacea* and *M. laeta* involve the structure of the thallus, pigmentation in the apothecia and spore size. *Micarea byssacea* usually produces the Sedifolia-grey pigment in apothecia, except when growing in deep shade. In addition, it forms a minutely granular thallus that is never areolate and has wider ascospores.

Micarea laeta, instead develops pale apothecia that are similar to *M. microareolata*. However, *M. microareolata* has narrower spores and an areolate thallus.

In the phylogenetic analysis, *M. microareolata* forms two subgroups differing by a small number of base pairs. The two subgroups show no morphological, chemical or ecological differences. In addition, a large quantity of data is missing, especially in the ITS regions of one subgroup. Therefore, at least for now, we treat these groups as one species instead of, for example, two closely related cryptic species.

Additional specimens examined. **Finland:** *Varsinais-Suomi*: Lohja, Ojamo, Ojamo lime quarry 200 m west, *Alnus glutinosa*/*Salix*-dominated swamp on shore of Lake Lohjanjärvi, on *Salix pentandra*, 33 m a.s.l., YKJ N6684589, E3335560 ±8m, 2014, *Pykälä* 47783 (H); *ibid*., on *Alnus glutinosa*, 32 m a.s.l., YKJ N6684555, E3335588 ±8m, 2014, *Pykälä* 47787 (H). *Pohjois-Karjala*: Lieksa, Koli National Park, E slope of Koli, old natural forest, on bark of fallen, decaying (late-stage) *Picea abies*, ETRS-TM35FIN N 7000213.0560, E 641998.5098, 2013, *Launis* 59133 (H); *ibid*., on bark of decaying (late-stage) *Betula* sp., ETRS-TM35FIN N7000159.5977, E642051.3884, 2013, *Launis* 89133 (H). *Etelä-Savo:* Joutsa, Höystösensuo, *Pinus sylvestris*-dominated mixed managed forest, on bark of standing decaying *Picea abies*, ETRS-TM35FIN E3459820, N6867631, 2015, *Launis* 186151 (H). *Varsinais-Suomi*: Lohja, Pappila, Tytyri lime quarry 150 m E, shore forest of Lake Lohjanjärvi, *Alnus*-dominated, on dead *Alnus glutinosa*, 32 m a.s.l., YKJ N6687374 E3338195 ± 8 m, 2015, *Pykälä* 47948 (H).—**Sweden:** *Östergötland*: Vadstena Region, Omberg, near top of Hjässan, well-lit forest, on bark of *Quercus robur*, 58°18'24·1"N, 14° 38'55·2"E, 262·8 m a.s.l., 2013, *Launis* 148131, 148132 (H).

Micarea pseudomicrococca Launis & Myllys sp. nov.

MycoBank No.: MB 824290

Thallus olive green, sometimes partly bright green, minutely granular, composed of goniocysts; apothecia abundant or few, 0·2–0·4 mm diam., plane, convex or ± hemispherical, sometimes becoming tuberculate, creamy-white or often pale brownish, always K− and C −; ascospores oblong-ellipsoid or obovoid, 0–1(–2)-septate, 8–14(–15) × 2·0–3·2 µm; methoxymicareic acid present. Resembles *M. micrococca* and *M. czarnotae.* Differs from *M. micrococca* by having an olive green instead of bright green thallus and thinner ascospores. Differs from *M. czarnotae* by forming less numerous and crowded apothecia, lacking the Sedifolia-grey pigment and forming a more granular thallus. In addition, *M. pseudomicrococca* has two types of paraphyses (up to 2 µm wide).

Type: Finland, Etelä-Häme, Jämsä, Hallinmäki Nature Reserve, *Betula* sp./*Picea abies*-dominated old-growth forest, on bark of decaying *Betula* stump, YKJ E3401759, N6894425, 2015, *Launis* 59151 (H—holotype). GenBank Accession numbers: ITS: MG521554, MG521555, MG521556. MtSSU: MG707755, MG707756, MG707757, MG707758. *Mcm7*: MG692516.

(Fig. 3G & H)

Thallus effuse, olive green, sometimes partly bright green, minutely granular, composed of goniocysts, 25–40(–55) µm diam., usually coalescing to form larger granules. *Photobiont* micareoid, algal cells 4·5–7·5 µm diam.

Apothecia abundant or few, 0·2–0·4 mm diam., plane, convex or ± hemispherical, sometimes becoming tuberculate, creamy-white or often pale brownish, always K− and C−. *Hypothecium* hyaline. *Hymenium* hyaline, sometimes with vertical brownish streaks, *c.* 35–50 µm high. *Epihymenium* hyaline or brownish. *Paraphyses* numerous, of two types: 1) scanty, scarcely branched, 0·8–1·0(–1·2) µm wide, apices usually not wider; 2) thicker, 1·2–2·0 µm wide with apices usually increasing up to 3 µm, simple or branched, sometimes branched 1–3 times from the apices resulting in a fork- or brush-like appearance. *Asci* clavate, *Micarea*-type, 8–10 × 30–35 µm. *Ascospores* oblong-ellipsoid or obovoid, 0–1(–2)-septate, 8–14(–15) × 2·0–3·2 µm.

Pycnidia of two types, cream-white or often brownish, always K− and C−. *Mesopycnidia* usually present and immersed in surrounding goniocysts, globose, up to 100 µm diam. *Mesoconidia* cylindrical or cylindrical-fusiform, 4·0–5·0 × 1·2–1·5 µm. *Micropycnidia* usually present, sometimes few or absent, sessile or immersed, if sessile usually with gaping ostiole, 80–100 µm diam. *Microconidia* filiform to narrowly fusiform, 5·5–9·0(–9·5) × 0·8–1·0(–1·2) µm.

Crystals (studied in polarized light). Visible in hymenium and in thallus. Soluble in K (Fig. 2E).

Chemistry. Methoxymicareic acid.

Etymology. The new species morphologically resembles a close relative, *M. micrococca*. The two species differ, however, in several anatomical features as well as phylogenetically.

Habitat and distribution. Collected on bark of *Betula* sp., *Prunus padus* and *Alnus incana*, and on decaying wood of fallen *Picea abies*. Known currently from Southern and Central Finland and from eastern Scotland.

Notes. Micarea pseudomicrococca is characterized by an olive green granular thallus and small creamy-white or pale brownish apothecia that lack the Sedifolia-grey pigment. In many respects it resembles the closely related species *M. micrococca* and *M. czarnotae*. These species are characterized by similar ecological preferences and the shape and size of the apothecia. In addition, all three species produce methoxymicareic acid.

The main morphological characters separating *M. pseudomicrococca* from *M. micrococca* and *M. czarnotae* involve the two types of paraphyses, structure and/or colour of thallus, pigmentation of apothecia and crystalline granules detectable in polarized light. *Micarea micrococca* forms a granular thallus, very similar in structure to *M. pseudomicrococca*, but the thallus of the latter is olive green instead of bright green. In addition, *M. micrococca* never develops brownish or greyish apothecia (Fig. 4B), its paraphyses are thinner and of one type instead of two, and it has wider ascospores. *Micarea czarnotae*, in contrast, forms numerous and often crowded apothecia and a less granular thallus compared to *M. pseudomicrococca*. It also produces the Sedifolia-grey pigment in the apothecia and no crystalline granules were detected in the thallus.

Additional specimens examined. **Finland:** *Pohjois-Karjala*: Lieksa, Koli National Park, E slope of Koli, old natural forest, on wood of decaying *Picea abies*, ETRS-TM35FIN N 7000159.5977, E 642051.3884, 2013, *Launis* 89132 (H). *Uusimaa*: Mäntsälä, Ohkolanjoki, *Picea abies*-dominated old-growth forest, by River Ohkolanjoki near railway, on bark of standing decaying (early-stage) *Alnus incana*, ETRS-TM35FIN N 6713368, E 399932, 2013, *Launis* 258131 (H). —**Great Britain:** *Scotland*: **V.C. 82,** East Lothian, Humbie, Church wood, on bark of *Prunus padus*, NT 46105, 64588, 2014, *Launis* 171141 & *Coppins* (H).

We wish to thank two anonymous reviewers for their useful and insightful comments which improved the text. We also warmly thank Dr Teuvo Ahti for valuable help with the nomenclature. Financial support for this study was provided by the research project "Conservation of wood-inhabiting Ascomycetes in changing forest landscapes" (grant number 7000T-YTB079), part of the research programme on insufficiently known and threatened forest species (PUTTE) financed by the Ministry of the Environment.

References

Andersen, H. L. (2004) *Phylogeny and classification of* Micarea. Ph.D. thesis, University of Bergen.

Andersen, H. L. & Ekman, S. (2005) Disintegration of the *Micareaceae* (lichenized *Ascomycota*): a molecular phylogeny based on mitochondrial rDNA sequences. *Mycological Research* **109:** 21–30.

Aptroot, A. & Cáceres, M. E. S. (2014) New lichen species from termite nests in rainforest in Brazilian Rondônia and adjacent Amazonas. *Lichenologist* **46:** 365–372.

Barton, J. & Lendemer, J. C. (2014) *Micarea micrococca* and *M. prasina*, the first assessment of two very similar species in eastern North America. *Bryologist* **117:** 223–231.

Brand, A. M., van den Boom, P. P. G. & Sérusiaux, E. (2014) Unveiling a surprising diversity in the lichen genus *Micarea* (*Pilocarpaceae*) in Réunion (Mascarenes archipelago, Indian Ocean). *Lichenologist* **46:** 413–439.

Brodo, I. M. (1984) The North American species of the *Lecanora subfusca* group. *Nova Hedwigia* **79:** 63–185.

Cáceres, M. E. S., Mota, D. A., de Jesus, L. S. & Aptroot, A. (2013) The new lichen species *Micarea corallothallina* from Serra da Jibóia, an Atlantic rainforest enclave in Bahia, NE Brazil. *Lichenologist* **45:** 371–373.

Coppins, B. J. (1983) A taxonomic study of the lichen genus *Micarea* in Europe. *Bulletin of the British Museum (Natural History), Botany Series* **11:** 17–214.

Coppins, B. J. (2002) *Checklist of Lichens of Great Britain and Ireland*. London: British Lichen Society.

Coppins, B. J. (2009) *Micarea* Fr. (1825). In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolseley, eds): 583–606. London: British Lichen Society.

Coppins, B. J. & Tønsberg, T. (2001) A new xanthone-containing *Micarea* from Northwest Europe and the Pacific Northwest of North America. *Lichenologist* **33:** 93–96.

Córdova-Chávez, O., Aptroot, A., Castillo-Camposa, G., Cáceres, M. E. S. & Pérez-Pérez, R. E. (2014) Three new lichen species from cloud forest in

Veracruz, Mexico. *Cryptogamie, Mycologie* **35:** 157–162.

Culberson, C. F. & Kristinsson, H. D. (1970) A standardized method for the identification of lichen products. *Journal of Chromatography A* **46:** 85–93.

Czarnota, P. (2007) The lichen genus *Micarea* (*Lecanorales*, Ascomycota) in Poland. *Polish Botanical Studies* **23:** 1–190.

Czarnota, P. & Guzow-Krzemińska, B. (2010) A phylogenetic study of the *Micarea prasina* group shows that *Micarea micrococca* includes three distinct lineages. *Lichenologist* **42:** 7–21.

Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32:** 1792–1797.

Ekman, S. & Svensson, M. (2014) *Brianaria* (*Psoraceae*), a new genus to accommodate the *Micarea sylvicola* group. *Lichenologist* **46:** 285–294.

Goloboff, P., Farris, J. & Nixon, K. (2008) TNT, a free program for phylogenetic analysis. *Cladistics* **24:** 774–786.

Guzow-Krzemińska, B., Czarnota, P., Łubek, A. & Kukwa, M. (2016) *Micarea soralifera* sp. nov., a new sorediate species in the *M. prasina* group. *Lichenologist* **48:** 161–169.

Hafellner, J. (1984) Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien *Lecanoraceae* und *Lecideaceae*. *Beiheft zur Nova Hedwigia* **79:** 241–371.

Hedlund, J. T. (1892) Kritische Bemerkungen über einige Arten der Flechtengattungen *Lecanora* (Ach.), *Lecidea* (Ach.) und *Micarea* (Fr.). *Bihang till Kongliga Svenska Vetenskaps-Akademiens Handlingar III* **18:** 1–104.

Kauff, F. & Lutzoni, F. (2002) Phylogeny of the *Gyalectales* and *Ostropales* (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetics and Evolution* **25:** 138–156.

Kirk, P. M., Cannon, P. F., Minter, D. W. & Stalpers, J. A. (2008) *Ainsworth & Bisby's Dictionary of the Fungi*, 10th edition. Wallingford: CABI Publishing.

Launis, A. & Myllys, L. (2014) *Micarea byssacea* new to North America and *M. hedlundii* new to Maine, Michigan and Quebec. *Opuscula Philolichenum* **13:** 84–90.

Leavitt, S. D., Johnson, L., Goward, T. & St. Clair, L. (2011) Species delimitation in taxonomically difficult lichen-forming fungi: an example from morphologically and chemically diverse *Xanthoparmelia* (*Parmeliaceae*) in North America. *Molecular Phylogenetics and Evolution* **60:** 317–332.

Lumbsch, H. T. & Leavitt, S. D. (2011) Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Fungal Diversity* **50:** 59–72.

Mark, K., Saag, L., Leavitt, S. D., Will-Wolf, S., Nelsen, M. P., Tõrra, T., Saag, A., Randlane, T. & Lumbsch, H. T. (2016) Evaluation of traditionally circumscribed species in the lichen-forming genus *Usnea*, section *Usnea* (*Parmeliaceae*, Ascomycota) using a six-locus dataset. *Organisms Diversity and Evolution* **16:** 497–524.

Maddison, D. R. & Maddison, W. P. (2005) *MacClade 4: Analysis of Phylogeny and Character Evolution*. Sunderland, Massachusetts: Sinauer Associates.

McCarthy, P. M. & Elix, J. A. (2016) A new species of *Micarea* (lichenized Ascomycota, *Pilocarpaceae*) from alpine Australia. *Telopea* **19:** 31–35.

Meyer, B. & Printzen, C. (2000) Proposal for a standardized nomenclature and characterization of insoluble lichen pigments. *Lichenologist* **32:** 571–583.

Myllys, L., Lohtander, K., Källersjö, M. & Tehler, A. (1999) Sequence insertion and ITS data provide congruent information in *Roccella canariensis* and *R. tuberculata* (*Arthoniales*, Euascomycetes) phylogeny. *Molecular Phylogenetics and Evolution* **12:** 295–309.

Myllys, L., Velmala, S., Holien, H., Halonen, P., Wang, L. S. & Goward, T. (2011) Phylogeny of the genus *Bryoria*. *Lichenologist* **43:** 617–638.

Orange, A., James, P. W. & White, F. J. (2010) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Schmitt, I., Crespo, A., Divakar, P. K., Fankhauser, J. D., Herman-Sackett, E., Kalb, K., Nelsen, M. P., Nelson, N. A., Rivas-Plata, E., Shimp, A. D., *et al*. (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* **23:** 35–40.

Schmull, M., Miadlikowska, J., Pelzer, M., Stocker-Wörgötter, E., Hofstetter, V., Fraker, E., Hodkinson, B., Reeb, V., Kukwa, M., Lumbsch, H. T., *et al*. (2011) Phylogenetic affiliations of members of the heterogeneous lichen-forming fungi of the genus *Lecidea sensu* Zahlbruckner (Lecanoromycetes, Ascomycota). *Mycologia* **103:** 983–1003.

Sérusiaux, E., Brand, A. M., Motiejūnaitė, J., Orange, A. & Coppins, B. J. (2010) *Lecidea doliiformis* belongs to *Micarea, Catillaria alba* to *Biatora* and *Biatora lignimollis* occurs in Western Europe. *Bryologist* **113:** 333–344.

Spribille, T., Klug, B. & Mayrhofer, H. (2011) A phylogenetic analysis of the boreal lichen *Mycoblastus sanguinarius* (*Mycoblastaceae*, lichenized Ascomycota) reveals cryptic clades correlated with fatty acid profiles. *Molecular Phylogenetics and Evolution* **59:** 603–614.

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30:** 1312–1313.

Svensson, M. & Thor, G. (2011) *Micarea capitata*, a new bryophilous lichen from Sweden. *Lichenologist* **43:** 401–405.

van den Boom, P. P. G. & Coppins, B. J. (2001) *Micarea viridileprosa* sp. nov., an overlooked lichen species from Western Europe. *Lichenologist* **33:** 87–91.

van den Boom, P. P. G. & Ertz, D. (2014) A new species of *Micarea* (*Pilocarpacea*) from Madeira growing on *Usnea*. *Lichenologist* **46:** 295–301.

van den Boom, P. P. G., Brand, A. M., Coppins, B. J. & Sérusiaux, E. (2017) Two new species in the

Micarea prasina group from Western Europe. *Lichenologist* **49:** 13–25.

White, T. J., Bruns, T., Lee, S. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. New York: Academic Press.

Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming Ascomycetes. *Lichenologist* **31:** 511–516.

https://doi.org/10.1017/S0024282918000555 Published online by Cambridge University Press